AR201-13660A

# The Flavor And Fragrance High Production Volume Consortia

## The Terpene Consortium

## Test Plan For Ionone Derivatives

**Methyl ionone (mixture of isomers)** **CAS No. 1335-46-2**

***alpha-iso*-Methylionone** **CAS No. 127-51-5**

## FFHPVC Terpene Consortium Registration Number



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# The HPV Challenge Test Plan for Ionone Derivatives

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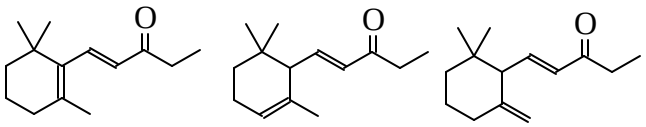
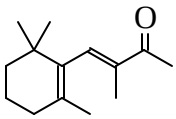


alpha beta gamma

**Methyl ionone (mixture of isomers)**
**$C_{14}H_{22}O$**

**Synonyms:**
1-Penten-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl (*alpha* methylionone isomer)
1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl (*beta* methylionone isomer)
1-Penten-3-one, 1-(6,6-methyl-2-methylenecyclohex-1-yl (*gamma* methylionone isomer)
3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- (*alpha-iso*-methylionone isomer)
3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (*beta-iso*–methylionone isomer)
3-Buten-2-one, 3-methyl-4-(6,6-dimethyl-2-methylenecyclohex-1-yl)- (*gamma-iso*-methylionone isomer)

**CAS No. 1335-46-2**



***alpha-iso*-Methylionone**
**$C_{14}H_{22}O$**

**Synonyms:**
3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
Isoraldeine
*alpha-iso*-Methylionone

**CAS No. 127-51-5**

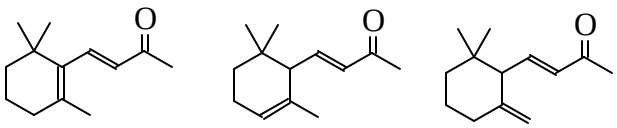
# 2 Category Analysis

## 2.1 Introduction

In October of 1999, members of the United States flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Terpene Consortium, as a member of the Flavor and Fragrance High Production Volume Consortia serves as an industry consortium to coordinate testing activities for terpenoid substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of The Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The test plan, category analysis, and robust summaries presented represent the first phase of the Consortiums commitment to the Chemical Right-to-Know Program.

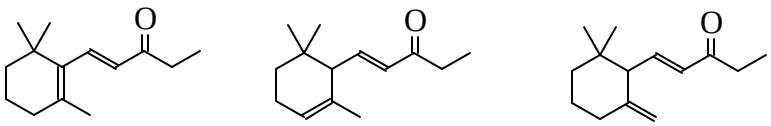
## 2.2 Background Information

The chemical category designated "Ionone Derivatives" includes two substances that are in reality a mixture of ionone isomers. Ionone, the parent terpene, occurs in nature as one of three isomers (*alpha, beta,* and *gamma*). Chemically, ionone is 4-(2,6,6-trimethylcyclohexen-1-yl)-2-buten-3-one. The three isomers differ only in the presence of a double bond at the 1, 2, or 2(exocyclic) position of the cyclohexane ring.



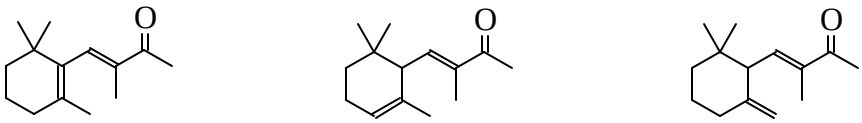
*alpha*-ionone *beta*-ionone *gamma*-ionone

Methylionone exhibiting the same double bond isomers as ionone contains an additional methyl group at the terminal position (4-position) of the butanone side chain.



*alpha*-methylionone *beta*-methylionone *gamma*-methylionone

*iso*-Methylionone exhibiting the same double bond isomers as ionone contains an additional methyl group at the 2-position of the butenone side chain.



*alpha*-*iso*-methylionone *beta*-*iso*-methylionone *gamma*-*iso*-methylionone

Ionones, methylionones and *iso*-methylionones are used as both fragrances and in food flavorings. *alpha*-Ionone and *beta*-ionone are a class of cyclic terpenoids occurring in essential oils exhibiting a sweet floral scent reminiscent of violets. Methylionones and *iso*-methylionone exhibit aromas associated with orris or violets.

## 2.3 Regulatory Status

A group of 21 ionone derivatives (Table 1) including the above substances have been reviewed by the Joint Expert Committee on Food Additives (JECFA) [JECFA, 1999] for use as flavoring substances in food. As part of its responsibility for the World Health Organization, JECFA maintains on ongoing program of review of the safety of flavor agents used as food additives (WHO Technical Series Nos. 38, 40, 42, 44). In 1999, these 21 ionone derivatives were recognized as safe under current conditions of use as flavoring substances added to food. These ionone derivatives are also recognized as Generally Recognized as Safe (GRAS) for their intended use in food by the United States Food and Drug Administration under the Code of Federal Regulations (CFR 172.515)

## Table 1. *alpha*- and *beta*-Ionone and Structurally Related Substances Used as Flavoring Substances

| SUBSTANCES | FEMA No. | CAS No. |
|---|---|---|
| 4-[(2,6,6)-Trimethyl-cyclohex-1-enyl] but-2-en-4-one (*beta*-Damascone) | 3243 | 23726-92-3 |
| *alpha*-Damascone | 3659 | 43052-87-5 |
| *delta*-Damascone | 3622 | 57378-68-4 |
| 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-2-en-4-one | 3420 | 23696-85-7 |
| 1,4-Dimethyl-4-acetyl-1-cyclohexene | 3449 | 43219-68-7 |
| *alpha* -Ionone | 2594 | 127-41-3 |
| *beta*-Ionone | 2595 | 14901-07-6 |
| *gamma*-Ionone | 3175 | 79-76-5 |
| *alpha* -Ionol | 3624 | 25312-34-9 |
| *beta*-Ionol | 3625 | 22029-76-1 |
| Dihydro-*alpha*-ionone | 3628 | 31499-72-6 |
| Dihydro-*beta*-ionone | 3626 | 17283-81-7 |
| Dihydro-*beta*-ionol | 3627 | 3293-47-8 |
| Dehydrodihydroionone | 3447 | 20483-36-7 |
| Dehydrodihydroionol | 3446 | 57069-86-0 |
| Methyl-*alpha*-ionone | 2711 | 127-42-4 |
| Methyl-*beta*-ionone | 2712 | 127-43-5 |
| Methyl-*delta*-ionone | 2713 | 7748-98-7 |
| Allyl-*alpha*-ionone | 2033 | 79-78-7 |
| *alpha*-Irone | 2597 | 79-69-6 |
| *alpha-iso*-Methylionone | 2714 | 127-51-5 |

## 2.4 Natural Occurrence

In plants, monoterpenoid ketones, such as *beta*-ionone, are formed by the biochemical oxidative cleavage of *beta*-carotene. The occurrence of *beta*-ionone in carrots arises from oxidation and cleavage of the 9'-10' double bond of *beta*-carotene. *beta*-ionone is also present as a minor metabolite in the animal metabolism of *beta*-carotene. In animals, *beta*-carotene is oxidized by carotenoid dioxygenase(s) and cleaved at the 15'-15' (central) double bond to yield two molecules of vitamin A (retinal) [Simpson and Chichester, 1981] which may be subsequently cleaved at the 9'-10' double bond to yield *beta*-ionone and 10'-apo-*beta*-carotenals. The presence of 10'-apo-*beta*-carotenal in rat liver following oral administration of *beta*-carotene is evidence that oxidative cleavage of the 9'-10' double bond occurs in animals [Sharma *et al*., 1977].

Ionone derivatives occur mainly in plants containing beta-carotene. They have been detected in a variety of foods including raspberries, carrots, roasted almonds, fruits and herbs [CIVO-TNO, 1999]. Eleven of the substances in the group of 21 have been reported to occur naturally in foods [CIVO-TNO, 1999]. Quantitative natural occurrence data and consumption ratios have been reported for 7 substances in the group and demonstrate that their consumption occurs predominantly from traditional foods (i.e., consumption ratio greater than 1) [Stofberg and Kirschman, 1985; Stofberg and Grundschober, 1987].

## 2.5 Structural Classification

The chemical category designated ionone derivatives includes *alpha-iso*-methylionone and a mixture of the *alpha*, *beta*, and *gamma* isomers of methylionone. Chemically, the only structural difference between *alpha-iso*-methylionone and the methylionone mixture is that *alpha-iso*-methylionone contains an additional methyl group at the 2-position of 4-(2,6,6-trimethylcyclohexen-1-yl)-2-buten-3-one (*alpha*-ionone) while methylionone contains an additional methyl group at the 4-position of 4-(2,6,6-trimethylcyclohexen-1-yl)-2-buten-3-one (*alpha*-ionone).

## 2.6 Industrial and Biogenic Production

### 2.6.1 Industrial Production

Methylionone derivatives are produced predominantly by the base catalyzed condensation of citral and methyl ethyl ketone. The reaction yields a mixture of *n*- and *iso*-psuedoionone, each of which may occur as four *cis-trans* isomers. The ratio of *n*- to *iso*-isomers depends on the condensation catalyst and reaction conditions. The normal (*n*-) isomers are favored when common alkaline catalysts are used. *Iso*-isomers are favored when strong alkaline catalysts such as quaternary ammonium bases are used [Beets and van Essen, 1959]. The preparation of the important fragrance *alpha-iso*-methylionone can be obtained in excellent yield from the reaction of dehydrolinalool with the enol ether of methyl ethyl ketone and methanol [Hoffman-LaRoche, 1961].

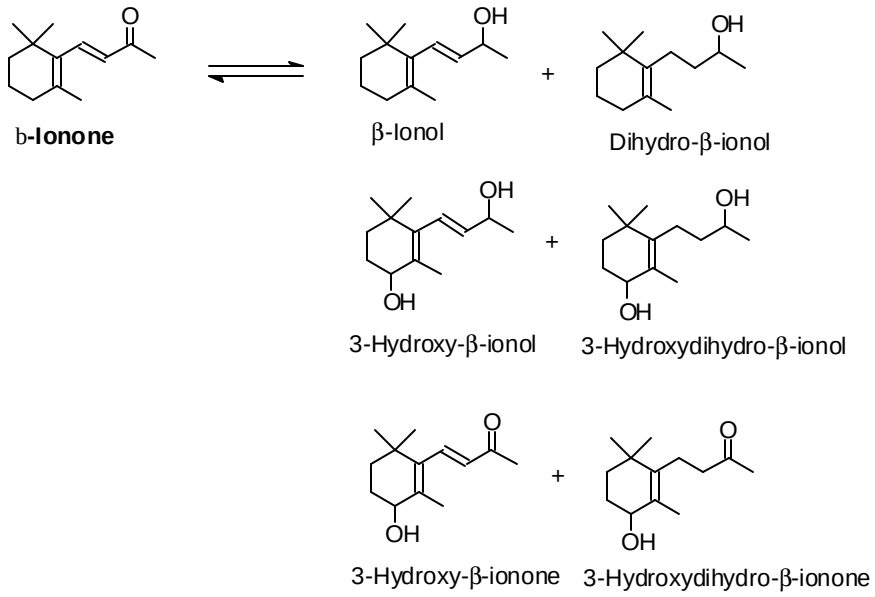
Cyclization of the open chain methylpseudoionones is accomplished with mineral acid (*e.g.*, sulfuric or phosphoric acid) and Lewis acid (*e.g.*, boron trifluoride etherate) catalysts. The ratio of *alpha*, *beta*, and *gamma* isomers of *iso*-methylionone and *n*-methylionone depends on the catalyst used and fluctuates from one manufacturer to another.

### 2.6.2 Chemical Reactivity and Metabolism

Orally administered ionones are absorbed and metabolized in mammals by allylic hydroxylation of the ring followed by oxidation of the hydroxyl group to 3-oxo derivatives. Reduction of the ketone function to the corresponding secondary alcohol also occurs. Combinations of these detoxication reactions result in the formation of multiple polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid (see Figure 1).

A total of 170 g *alpha*-ionone was fed to 2 rabbits over an unspecified period. Analysis of collected urine revealed a hydroxylated derivative of *alpha*-ionone [Prelog and Wursch, 1951] formed from allylic ring oxidation.

**FIGURE 1. METABOLISM OF *BETA*-IONONE IN RABBITS**




A 3 kg male rabbit was orally administered a total of 23 g *beta*-ionone for 7 days (approx. 1000 mg/kg bw/day). Urine was collected daily and for 4 days after the final dose. Allylic ring oxidation and ketone reduction yielded 3-oxo-ionone, 3-oxo-*beta*-ionol, dihydro-3-oxo-*beta*-ionol, and 3-hydroxy-*beta*-ionol, which were detected in the urine. Unchanged *beta*-ionone and the glucuronic acid conjugates of 3-oxo- *beta*-ionol and dihydro-3-oxo-*beta*-ionol were also detected [Ide and Toki, 1970] (see Figure 1). Two rabbits received a total of 100 g *beta*-ionone over 18 days via esophageal tube. Metabolites identified in urine, which was collected throughout the treatment period, included 3-hydroxy-*beta*-ionone, 3-oxo-*beta*-ionol, and 3-hydroxy-*beta*-ionone[1]. A hydroxyketone thought to be either 3-oxo-*beta*-ionol or 3-hydroxy-*beta*-ionone was also recovered [Fujii *et al.*, 1972].

The exocyclic dihydro metabolites of *beta*-ionone and *beta*-ionol have also been detected in rabbits. *beta*-Ionone in 20% alcohol was orally administered to 3 rabbits in doses of 2000 to 5000 mg/day for 2 weeks. Analysis of urine revealed *beta*-ionol, *beta*-ionone,

[1] Urinary metabolites are identified here using IUPAC nomenclature. Metabolites are reported in the reference using a different naming system.

dihydro-*beta*-ionol, 3-hydroxy-*beta*-ionol, 3-hydroxy-dihydro-*beta*-ionol, 3-hydroxy-*beta*-ionone, and 3-hydroxy-dihydro-*beta*-ionone[1] [Bielig and Hayasida, 1940] (see Figure 1). Two dogs fed a total of 100 g *beta*-ionone over 18 days excreted 3-oxo-*beta*-ionone and 3-hydroxy-*beta*-ionol in the urine [Prelog and Meier, 1950].

*beta*-Ionone has been found to induce biphenyl 4-hydroxylase, glucuronyl transferase, 4-nitrobenzoate reductase, and cytochrome P-450 in rats following 3-day administration via either intraperitoneal injection or food [Parke and Rahman, 1969].

The metabolism of ionones is expected to be similar in humans. This is supported by human metabolism studies of retinoids and carotenoids such as *cis*-13-retinoic acid (i.e., isotretinoin) and *beta*-carotene, respectively, which possess ionone fragments. The primary blood and biliary metabolites following oral administration of isotretinoin to humans include the glucuronic acid conjugates of isotretinoin [Kraft *et al.*, 1991] and the allylic oxidation product [Vane *et al.*, 1990; Kraft *et al.*, 1991]. Both metabolites were observed in the blood and bile of cynomologous monkeys provided in isotretinoin via the oral route [Kraft *et al.*, 1991]. Allylic hydroxylation of the methyl ring substituent and subsequent conjugation with glucuronic acid also occurs in humans [Vane *et al.*, 1990].

# 3 Test Plan

## 3.1 Chemical and Physical Properties

### 3.1.1 Melting Point

The two substances in this chemical category are liquids at ambient temperature. Calculated value for *alpha-iso*-methylionone and methylionone (mixture of isomers) are 45.26 $^{o}$C and 59.38 $^{o}$C, respectively (mean value) [MPBPWIN EPI Suite, 2000].

### 3.1.2 Boiling Point

Literature values are available for *alpha-iso*-methylionone and methylionone (mixture of isomers). The Fragrance Materials Association (FMA) has reported that *alpha-iso*-methylionone and methylionone (mixture of isomers) both exhibit a boiling point of 238 $^{o}$C @ 760 mm [FMA]. The experimental boiling point reported for *alpha-iso*-methylionone is 266.2 $^{o}$C @ 749 mm (1013 Pa), 162.2 $^{o}$C @ 39 mm Hg (53.3.Pa), and 126.5 $^{o}$C @ 10 mm Hg (13.3 Pa) [Hoffmann-LaRoche, Inc., 2000]. The calculated values [MPBPWIN EPI Suite, 2000] for *alpha-iso*-methylionone and methylionone (mixture of isomers) of 271.6 $^{o}$C and 274.6 $^{o}$C (adapted Brown and Stein Method) are in good agreement with measured values given that boiling points were measured for mixtures of isomers.

### 3.1.3 Vapor Pressure

The calculated vapor pressure value [MPBPWIN EPI Suite, 2000] is in the range from 0.00146-0.00150 kPa (0.0124 to 0.0127 mm Hg) (modified Grain method) for *alpha-iso*-methylionone based on an experimental boiling point of 266.2 $^{o}$C [Hoffmann-LaRoche, 2000]. The calculated vapor pressure value [MPBPWIN EPI Suite, 2000] is estimated to be 0.00124 kPa (0.0093 mm Hg) (modified Grain method) for methylionone mixture based on the same experimental boiling point [Hoffmann-LaRoche, 2000]. These

calculated values are in good agreement with vapor pressure values reported by the Fragrance Materials Association. The calculated vapor pressures for *alpha-iso*-methylionone and methylionone mixture are 0.006 and 0.005 mm Hg, respectively [FMA, 2000]. The range of vapor pressure at ambient temperature is fairly narrow. The vapor pressure for ionone derivatives in this category are in the range from 0.009 to 0.013 mm Hg. Based on the close agreement among these values, no further testing is recommended.

### 3.1.4 n-Octanol/Water Partition Coefficient

The measured log Kow values for methyl ionone mixture containing 87.8% *alpha-iso*-methylionone is 4.6 using an OECD 117 test protocol [Rudio, 1994a]. This is in good agreement with the measured log Kow of 4.1 for lower molecular weight ionone *beta*-ionone using the same OECD 117 protocol performed at the same laboratory [Rudio, 1994b]. The measured log Kow value for *alpha-iso*-methylionone is also in good agreement with the calculated values of log Kow values of 4.84 for both members of this chemical category. [KOWWIN EPI Suite, 2000]. The narrow range and the close agreements with the one measured value and the calculated values indicate consistency and imply reliability. Based on the mutual agreement of these values, no further partition coefficient studies are recommended.

### 3.1.5 Water Solubility

The reported water solubility for *alpha-iso*-methylionone is 16 mg/L at 20 $^{o}$C following an OECD 105 protocol for determination of water solubility [Schlienger, 1992b]. A measured value of 90 mg/L was reported for *gamma*-methylionone but the temperature was specified [BBA, 1990]. The calculated value [WSKOWIN EPI Suite, 2000] for *alpha-iso*-methylionone or methylionone mixture is 4.8 mg/L at 25 $^{o}$C. The solubility of the two members of this chemical category at 20 $^{o}$C is expected to be in the range from approximately 15 mg/L. No further solubility studies are recommended.

### 3.1.6 New Testing Required

None.

## 3.2 Environmental Fate and Pathways

### 3.2.1 Photodegradation

The calculated photodegradation half-lives [AOPWIN EPI Suite, 2000] for the reaction of structurally defined substance *alpha-iso*-methylionone with hydroxyl (OH) radicals or ozone ($O_3$) is 0.75 or 0.5 hours, respectively. These calculations are based on measured rate constants for *alpha-iso*-methylionone, but would expected to be the same for the methylionone mixture based on the presence of the *alpha-iso*-methyl isomer in the mixture and the close structural relationship among all members of this category.

### 3.2.2 Stability In Water

No hydrolysis is possible for any of the materials in this group. All are expected to be very stable in aqueous solution.

### 3.2.3 Biodegradation

Five GLP experimental studies evaluating biodegradability are available for this group of substances using standard OECD protocols. Three studies on methylionone isomers showed ready biodegradability. The first, which followed a MITI OECD 301C protocol, reported 70.5% biodegradation at 28 days with 10% biodegradation reached within 5 days [Calame and Ronchi, 1990]. The second study on methylionone, which followed a Method F protocol and evaluated ready biodegradability, reported 99.1% biodegradation at 31 days [Stickley, 1990]. In the third test using a respirometric method, methylionone was reported to be 80% degraded within 28 days [Givaudan-Roure, 1991].

Two biodegradability tests have been performed for *alpha-iso*-methylionone. In the first test using a Modified MITI OECD 302C protocol, *alpha-iso*-methylionone exhibited primary inherent biodegradability of 63.4% after 56 days and was slightly toxic to microorganisms [Schlienger, 1992a]. In a later test using an OECD 301B protocol, *alpha-iso*-methylionone was considered as inherently biodegradable under modified ready test conditions (61.8% after 28 days) [King, 1992]. Given the close structural relationship between *alpha-iso*-methylionone and methylionone and the fact that the methylionone mixture contains significant amounts of the *alpha-iso*-methyl isomer, it is unlikely the two substances should exhibit any significant difference in biodegradability according to standardized protocols. The results of the five studies support that conclusion. Therefore, it is likely that members of this chemical category will exhibit ready biodegradability.

### 3.2.4 Fugacity

Transport and distribution in the environmental were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Trent University, 1999]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. Where measured values were available, these were used, but where they were not, calculated data from the EPIWIN series of programs were used. Given the similarity of structure and physical properties of the substances in this category, they would be predicted to exhibit similar distribution in the environment. The value of these calculations must be evaluated in the context that the substances in this chemical category are products of plant biosynthesis and are, therefore, ubiquitous in the environment. Also, the models fail to account for the known chemical reactivity of these substances.

### 3.2.5 New Testing Required

None.

## 3.3 Ecotoxicity

### 3.3.1 Acute Toxicity to Fish

The two substances, *alpha-iso*-methylionone and *beta*-ionone have measured fish acute toxicity data [Schlienger, 1992b; Grothe, 1989]. The 96-hour LC50 of 10.9 mg/L for *alpha-iso*-methylionone in rainbow trout (*Oncorhynchus mykiss*) agree with limit data for *beta*-ionone in the same species [Grothe, 1989]. The LCO value of 5.0 mg/L [Grothe, 1989] for *beta*-ionone indicates that the acute LC50 for rainbow trout is in the range of 10 mg/L. The calculated LC50 of less than 1 mg/L for *alpha-iso*-methylionone is estimated as a neutral organic and 2-3 mg/L estimated as vinyl/allyl ketone demonstrates the conservative nature of the ECOSAR model [ECOSAR EPI Suite, 2000]. Based on the experimental data, the two ionone derivatives exhibit low acute toxicity to fish.

### 3.3.2 Acute Toxicity to Aquatic Invertebrates

The principal substance in this group, *alpha-iso*-methylionone exhibits a 48-hour LC50 of 0.6 mg/L in *Daphnia magna* calculated both as a neutral organic and a vinyl/allylketone [ECOSAR EPI Suite, 2000]. In order to validate the model, it is recommended that an acute LC50 in *Daphnia magna* should be determined for *alpha-iso*-methylionone using an OECD 202 guideline.

### 3.3.3 Acute Toxicity to Aquatic Plants

The principal substance in this group, *alpha-iso*-methylionone exhibits a calculated 48-hour EC50 of 0.332 or 0.266 mg/L in *Daphnia magna* calculated both as a neutral organic and a vinyl/allylketone [ECOSAR EPI Suite, 2000]. In order to validate the model, it is recommended that an acute EC50 in algae should be determined for *alpha-iso*-methylionone using an OECD 201 guideline.

### 3.3.4 New Testing Required

- An acute LC50 for *alpha-iso*-methylionone is recommended in *Daphnia magna* using OECD 202 Guideline.
- An acute EC50 for *alpha-iso*-methylionone is recommended in algae using OECD 201 Guideline.

## 3.4 Human Health Toxicity

### 3.4.1 Acute Toxicity

Rat oral LD50 values available for *alpha*-ionone, *alpha-iso*-methylionone, methylionone (mixture of isomers), and *gamma*-ionone indicate these materials to be very low in oral acute toxicity. The LD50 values of all four substances are greater than 5000 mg/kg bw [Moreno, 1973a, 1973b, 1977a, 1977b]. Mouse oral LD50 values are also greater than 5000 mg/kg bw for *alpha*-ionone, *alpha-iso*-methylionone, and methyl ionone (mixture of isomers). The mouse oral LD50s are reported to be 6650 mg/kg bw for *alpha*-ionone, 5331 mg/kg bw for methylionone, and 8714 for *alpha*-isomethylionone [Hoffman LaRoche, 1967]. The acute dermal LD50s reported for *alpha-iso*-methylionone, methylionone (mixture of isomers), and *gamma*-ionone are all greater than 5000 mg/kg bw [Moreno, 1973a, 1977a, 1977b]. Based on these results the acute oral and dermal toxicities of ionone derivatives is concluded to be very low.

### 3.4.2 Genetic Toxicity

#### 3.4.2.1 *In vitro* Genotoxicity

*In vitro* genotoxicity assays available for various ionone and methyl ionone isomers demonstrate that these substances have a little, if any, genotoxic potential. *beta*-Ionone exhibited no mutagenic activity in established strains of *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) at concentrations up to approximately 180 µg/plate

with and without metabolic activation [Mortlemans *et al.*, 1986; Florin *et al.*, 1980]. Two methylionone isomers, *alpha*- and *delta*-methylionone exhibited no mutagenic activity in *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538 and TA1598 with and without S-9 activation, at concentrations up to approximately 3600 µg/plate [Wild *et al.*, 1983]. No mutagenic activity was observed in *Salmonella typhimurium* strains TA98 and TA100 when treated with *alpha*-ionone at concentrations up to approximately 50 µg/plate with and without metabolic activation [Kasamaki *et al.*, 1982]. In recent study [Wagner and Caruthers, 1999], there is no evidence of mutagenicity or precipitation at concentrations up to and including 5000 ug/plate when methylionone was incubated with Salmonella typhimurium TA100, TA98, TA1535, and TA1537. Toxicity was observed at concentrations of 1800 ug/plate with TA100 and 1800 ug/plate with TA1537. In a chromosome aberration test using Chinese hamster cells, line B241, *alpha*-ionone was positive at a concentration of 25 mM (5150 ug/ml). However, this study must be interpreted with caution since the investigators made no attempt to monitor cytotoxicity in CHO cells [Kasamaki *et al.*, 1982].

#### 3.4.2.2 *In vivo* Genotoxicity

No increase in the frequency of sex-linked recessive mutations occurred when *Drosophila melanogaster* were maintained on 20 mM solutions of *alpha*-methylionone [Wild *et al.*, 1983].

In a clastogenicity assay, groups of 10- to 14-week-old NMRI mice were intraperitoneally injected at 0 and 24 hours with 333, 666, or 1,000 mg/kg bw of *alpha*-methylionone [Wild *et al.*, 1983]. At 30 hours, the mice were sacrificed and bone marrow smears were prepared using the staining method of Schmid (1976). There was no evidence of micronucleated polychromatic erythrocytes for treated or control groups (mean number of micronucleated PE/1000 NCE at 0, 825,1444 or 2063 mg/kg bw was 1.7, 1.0, 0.7, or 1.9, respectively).

Based on the results of this *in vivo* genotoxicity assays and the lack of any evidence of genotoxicity for numerous *in vitro* assays with and without metabolic activation, it is

unlikely that any of these materials would exhibit a significant genotoxic potential *in vivo*. No additional *in vitro* and *in vivo* assays are requested for this chemical category.

### 3.5 Repeat Dose Toxicity

Ninety (90) day dietary studies have been performed with *alpha-iso*-methylionone *alpha*-ionone, *beta*-ionone in both sexes of rats [Oser *et al.*, 1965]. Groups of 15 FDRL rats (per sex per dose) were housed individually and maintained on a diet containing the test article diluted in cotton-seed oil (2%), a concentration sufficient to provide an average daily intake of 3.55 or 4.10 mg/kg bw of *alpha*-iso-methylionone for males and females, respectively. Animals were housed individually. Weekly measurement of body weights and food and water intake revealed no significant differences between test and control animals. Hematological examination and blood chemical determinations performed on 8 rats at week 6 and on all rats at week 12 showed normal values. Measurement of liver and kidney weights at necropsy showed no differences in absolute or relative organ weights between test and control groups. Histological examination was performed on the adrenal, bladder, brain, bone marrow, heart, ileum, kidney, liver, lung, lymph nodes, mammary, salivary glands, ovary, pancreas, pituitary, thyroid, large intestines, spinal cord, spleen, stomach and testis. Based on gross and histopathological examination, there were no alterations that could be associated with administration of *alpha-iso*-methylionone.

In the two other studies using the same protocol, no evidence of toxicity was reported when groups of male and female FDRL rats were maintained on diets calculated to result in an average daily intake of 11.8 or 10.6 mg/kg bw of *alpha*-ionone or 11.6 or 11.4 mg/kg bw of *beta*-ionone, respectively, for 90 days, [Oser *et al.*, 1965].

In two other dietary studies, groups of Sprague-Dawley rats (15/sex/group) housed in groups of 3 were maintained on diets calculated to contain 10 or 100 mg/kg bw of *alpha*-ionone or *beta*-ionone for 90 days. Body weights and food and water intake were measured every 3rd or 4th day of the study. Hematological examination was performed on rats during weeks 6 and 13 of the study. Blood chemical determinations and urinanalysis were performed on weeks 5 and 12. At necropsy, organ weights (brain, liver,

spleen, kidneys, caecum, adrenals and gonads (males)) were measured. Histopathological examination of a wide variety of tissues (adrenal, aorta, bladder, brain, caecum, colon, diaphragm, duodenum, epididymis, eye, harderian gland, heart, ileum, kidney, liver, lung, lymph nodes, mammary, muscle, esophagus, ovary, pancreas, pituitary, prostate, rectum, seminal vesicles, skin, spinal cord, spleen, stomach and testis) were performed for the controls and high dose groups. The liver of the low dose group was also subjected to histopathological examination.

In the *alpha*-ionone study, actual intake was determined to be 11 mg/kg bw for males and females at the 10 mg/kg bw target dose and 106-108 mg/kg bw at the 100 mg/kg bw target dose. Food intake of the high dose group of males and females were significantly lower than controls. A decrease in neutrophils and lymphocytes were reported in males at the high dose level at week 6 but not at week 13. At the high dose, lower alkaline phosphatase in males and lower glucose levels in females was reported. The relative kidney weights were statistically significantly greater in males at the high dose. Relative and absolute mean liver weights were statistically increased in males at the high dose. The only histological finding was desquamation of the thyroid in females only at the high dose. The NOAEL for *alpha*-ionone was reported to be 10 mg/kg [Gaunt *et al*., 1983].

In the *beta*-ionone study, the actual intake of *beta*-ionone was determined to be 11 mg/kg bw for males and females at the 10 mg/kg bw target dose and 106-108 mg/kg bw at the 100 mg/kg bw target dose. Food intake of the high dose group of males and females were significantly lower than controls. A decrease in erythrocyte counts and hematocrit were reported in males at the high dose levels at week 6 but not at week 13. At the high dose, lower alkaline phosphatase in males and lower glucose levels in females was reported. Relative and absolute mean liver weights were statistically increased in males at the high dose. Relative brain, caecal, liver and kidney weights were statistically increased in females at the high dose level. The NOAEL for *beta*-ionone was reported to be 10 mg/kg [Gaunt *et al*, 1983].

In a dermal 90-day study with one of the methylionone isomers (*gamma*-methylionone), a 1% *gamma*-methylionone solution in phenethyl alcohol at a dose of 10 mg/kg was

applied topically to the clipped backs of individually housed Sprague-Dawley rats (5/sex/group) daily for 90 days. A control group of 5 male and female rats received 1 ml/kg phenyl ethyl alcohol. Body weights were measured weekly. Hematological examination, clinical chemistry determinations and urinalysis were performed on all animals at termination. At necropsy, liver and kidney weights were measured and histopathological examination was made of the skin, kidneys, liver, sternal bone, and spinal cord. There was no evidence of toxicity induced by treatment with the *gamma* methyl ionone [Moreno, 1981].

Based on the results of dietary studies with *alpha-iso*-methylionone, *alpha*-ionone, and *beta*-ionone [Oser *et al.*, 1965; Gaunt *et al*, 1983] and a dermal study with *gamma*-methylionone, it is concluded that none of the ionone derivatives exhibits any evidence of toxicity at dose levels up to and including 10 mg/kg bw/day.

### 3.5.1 Reproductive Toxicity

The effect of ionone (*alpha*- and *beta*-ionone) on the reproduction in 48 white rats was investigated. The females received 0.1 ml oil solution containing 2 mg ionone by gavage every other day for 8 months. Males were also given 2mg/day every other day for 8 months. The dose corresponds to a daily intake of approximately 8 to 10 mg/kg bw/day. Females were followed through 3 reproduction cycles. Females were monitored for number of pregnancies, average weight, number of born offspring, number of offspring born alive, weight at birth and after 7 and 21 days, and viability of offspring after each reproduction. Females received 24 mg before the first reproduction, 84 mg before the second, and 208 mg before the third reproduction. Offspring from the first reproduction (F1) were allowed to reach maturity. This F1 generation received 15 mg ionone prior to reproduction. The F1 generation was then monitored for the same parameters as for females above. Based on data collected from three reproductive cycles of female rats and on reproductive cycle of F1 offspring given 2 mg/day every other day (approximately 8-10 mg/kg bw per day) by gavage, there is no evidence of reproductive toxicity [Sporn *et al.*, 1965].

The lack of reproductive toxicity [Sporn *et al.*, 1965] is supported by observations made in two separate repeat dose studies [Oser *et al.*, 1965; Gaunt *et al*, 1983] in which there was no evidence (organ weight, gross or histopathological) toxicity to reproductive organs (testis, seminal vesicles, ovaries) of rats maintained on diets of up to 100 mg/kg bw/day for 90 days. Therefore, it is concluded that none of the ionone derivatives discussed show any evidence of reproductive toxicity.

### 3.5.2 Developmental/Teratogenicity Toxicity

Groups of pregnant LAK:LVG(SYR) hamsters were given 0, 48, 240, or 480 mg/kg bw of *beta*-ionone dissolved in acetone (5%) and solubilized in Tween 20 by gavage on day 8 of pregnancy. The low- mid-, and high-dose group contained 6, 9, and 14 animals. The doses were chosen based on the median effective dose of retinoids that induce terata (ED50) in hamsters. Animals were sacrificed on day 14 and average fetal and maternal body weights were measured. Developmental parameters monitored included number of litters, abnormal litters, implantation sites, number resorptions, number of abnormal live fetuses, number dead fetuses, mean litter frequency, and characterization of malformations. The only malformation recorded was that one fetus at the 480 mg/kg bw dose level exhibited one hind limb lateral rotation. The authors reported concluded that this effect was not associated with administration of the test substance. The authors concluded that dose levels up to and including 480 mg/kg bw of *beta*-ionone failed to show any evidence of maternal or developmental toxicity in golden Syrian hamsters [Willhite, 1986]. In the same study, dose levels of 96 or 960 mg/kg bw of psuedoionone (a structurally related ketone) were given to golden Syrian hamsters on day 8 of pregnancy. The only effect reported was a significant reduction in maternal weight gain in the 960 mg/kg bw group. There were no developmental effects at either dose level [Willhite, 1986].

### 3.5.3 New Testing Required

None.

## 3.6 Test Plan Table

| Chemical | Chemical and Physical Properties | | | | |
|---|---|---|---|---|---|
| | **Melting Point** | **Boiling Point** | **Vapor Pressure** | **n-Octanol/Partition Coefficient** | **Water Solubility** |
| **CAS No. 127-51-5** *alpha-iso*-Methylionone | Calc | A, Calc | A, Calc | A, Calc | A, Calc |
| **CAS No. 1335-46-2** Methyl ionone (mixture of isomers) | Calc | A, Calc | A, Calc | R, Calc | A |

| Chemical | Environmental Fate and Pathways | | | |
|---|---|---|---|---|
| | **Photodegradation** | **Stability in Water** | **Biodegradation** | **Fugacity** |
| **CAS No. 127-51-5** *alpha-iso*-Methylionone | Calc | Calc | A | Calc |
| **CAS No. 1335-46-2** Methyl ionone (mixture of isomers) | R | NA | A, R | Calc |

| Chemical | Ecotoxicity | | |
|---|---|---|---|
| | **Acute Toxicity to Fish** | **Acute Toxicity to Aquatic Invertebrates** | **Acute Toxicity to Aquatic Plants** |
| **CAS No. 127-51-5** *alpha-iso*-Methylionone | A, Calc | Test, Calc | Test, Calc |
| **CAS No. 1335-46-2** Methyl ionone (mixture of isomers) | R, Calc | Calc | Calc |

| Chemical | Human Health Toxicity | | | | | |
|---|---|---|---|---|---|---|
| | **Acute Toxicity** | **Genetic Toxicity *In Vitro*** | **Genetic Toxicity *In Vivo*** | **Repeat Dose Toxicity** | **Repro-ductive Toxicity** | **Develop-mental Toxicity** |
| **CAS No. 127-51-5**<br>*alpha-iso*-Methylionone | A | A | R | A | R | R |
| **CAS No. 1335-46-2**<br>Methyl ionone (mixture of isomers) | A | A | A | A, R | R | R |

| Legend | |
|---|---|
| **Symbol** | **Description** |
| R | Endpoint requirement fulfilled using category approach, SAR |
| Test | Endpoint requirements to be fulfilled with testing |
| Calc | Endpoint requirement fulfilled based on calculated data |
| A | Endpoint requirement fulfilled with adequate existing data |
| NR | Not required per the OECD SIDS guidance |
| NA | Not applicable due to physical/chemical properties |
| O | Other |

# 4 References for Test Plan and Robust Summaries


AOPWIN EPI Suite (2000) US Environmental Protection Agency.

Beets M.G.J. and van Essen, H. (1959) Polak & Schwarz International NV, GB 812 727. *Chem. Abstr.*, **53**, 22067f.

Bielig H. and Hayasida A. (1940) Uber die verhalten des *beta*-jonone im tierkorper (biochemische hydrietungen VIII). Hoppe-Seyler's Zeitschr. *Physiol. Chem*. **266**, 99-111.

Bush, Boake, Allen, Inc. (BBA) (1990) Biodegradability of *p-t-butyl-alpha*-methylhydrocinnamic aldehyde and methyl-*alpha*-ionone. Unpublished report.

Calame R. and Ronchi W. (1990) Isoraldeine 70. Determination of ready biodegradability. Report No. 90-42/B. Unpublished report.

CIVO-TNO (1999) *Volatile Components in Food-Qualitative and Quantitative Data.* Supplement 5 to the 6th Edition. Edited by H. Maarse, C.A. Visscher, L.C. Willemsens, L.M. Nijssen, and M.H. Boelens. TNO Nutrition and Food Research. Zeist, The Netherlands.

ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Florin I., Rutberg, L., Curvall, M., and Enzell, C. R. (1980) Screening of Tobacco Smoke Constituents for Mutagenicity Using the Ames' Test. *Toxicology*, **18**, 219-232.

Fragrance Materials Association (FMA) Unpublished report.

Fujii T., Furukawa S., and Suzuki S. (1972) Compounded perfumes for toilet goods. Non-irritative compounded perfumes for soaps. *Yukagaku*, **21**, 904-908.

Gaunt I. F., Butler, W., Ford, G. (1983) The short-term (90 Days) toxicity of *alpha* and *beta*-Ionones in rats. Unpublished report to IOFI.

Givaudan-Roure Inc. (1990) Determination of the ready biodegradability of methylionone. Unpublished report.

Givaudan-Roure Inc. (1991) Biodegradability test of ionone, *beta*, synthetic. Report No. 5992503. Unpublished report

Givaudan-Roure Inc. (1994) Partition Coefficient *n*-octanol/water of methyl ionone. Unpublished report.

Grothe J. (1989) Ecotoxicity attachment for beta-ionone. Roche report No. E-29/89. Unpublished report.

Hoffmann-LaRoche, Inc. (1961) AD, GB 865 478. *Chem. Abstr.*, **55**, 20996a.

Hoffmann-LaRoche, Inc. (1967) Acute toxicity, eye and skin irritation test on aromatic compounds. Unpublished report.

Hoffmann-LaRoche, Inc. (2000) Isoraldeine 70 Safety Data Sheet. Red Corner Report, No. B-108,080 vom 17.6., 1983, Kradolfer (Nr. 95931). Unpublished report.

Ide H. and Toki S. (1970) Metabolism of *beta*-ionone. Isolation, characterization and identification of the metabolites in the urine of rabbits. *Biochemical Journal*, **119**, 281-287.

JECFA (1999) Evaluation of certain food additives. Joint FAO/WHO Expert Committee on Food Additives. World Health Organization. Technical Report Series No. 42, pp 335-352.

Kasamaki A., Takahashi, H., Tsumura, N., Niwa, J., Fujita, T. and Urasawa, S. (1982) Genotoxicity of Flavoring Agents. *Mutation Research*, **105**, 387-392.

King J. M. (1992) The inherent biodegradability of base perfumes in the sealed vessel test. BD/PER/15. Unpublished report.

KOWWIN EPI Suite (2000) 2000 US Environmental Protection Agency.

Kraft J.C., Slikker Jr. W., Bailey J.R., Roberts L.G., Fischer B., Wittfoht W. and Nau H. (1991) Plasma pharmacokinetics and metabolism of 13-cis- and all-trans-retinoic acid in the cynomolgus monkey and the identification of 13-cis- and all-trans-retinoyl- *beta*-glucuronides. A comparison to one human case study with isotretinoin. *Drug Metabolism and Disposition*, **19**, 317-324

Moreno O. M. (1973a) Acute toxicity studies on rats and rabbits. Unpublished report to RIFM.

Moreno O. M. (1973b) Acute toxicity studies on rats and rabbits. Unpublished report to RIFM.

Moreno O. M. (1977a) Acute toxicity studies on rats and rabbits. Unpublished report to RIFM.

Moreno O. M. (1977b) Acute toxicity studies on rats and rabbits. Unpublished report to RIFM.

Moreno O. M. (1981) 90-Day sub acute dermal toxicity in rats. Unpublished report to RIFM.

Mortelmans K., Haworth, S., Lawlor, T., Speck, W., Tainer, B and Zeiger, E. (1986) Salmonella Mutagenicity Tests: II. Results from the Testing of 270 Chemicals. *Environmental Mutagenesis*, **8(Supp. 7),** 1-119.

MPBPWIN EPI Suite (2000) US Environmental Protection Agency.

Oser B. L., Carson S. and Oser M. (1965) Toxicological tests on Flavor Matters. *Food and Cosmetic Toxicology,* **3**, 563-569.

Parke D.V. and Rahman H. (1969) The effects of some terpenoids and other dietary anutrients on hepatic drug-metabolizing enzymes. *Biochemical Journal,* **113**, 124.

Prelog V. and Meier H.L. (1950) Uber die biochemische oxydation von *beta*-ionon im tierkorper. *Helvetica chimica acta,* **33**, 1276-1284.

Prelog V. and Wursch J. (1951) Uber die biochemische oxydation von *alpha*-ionon im tierkorper. *Helvetica chimica acta,* **34**, 859-861.

Rudio J. (1994a) Partition coefficient n-octanol/water of Isoraldeine according to OECD Guideline No. 117. Study No. 94-E70. Unpublished report.

Rudio J. (1994b) Partition coefficient n-octanol/water of ionone, beta synt according to OECD Guideline No. 117. Study No. 94-E68. Unpublished report.

Schlienger C. (1992a) Inherent biodegradability: Modified MITI-TEST (II) for Isoraldein 70. GLP Test No. PSU 92/2-MII. Unpublished Report.

Schlienger C. (1992b) 96-Hour acute toxicity study with Isoraldein 70 in rainbow trout. Report No. B-161751. Unpublished report.

Sharma R.V., Mathur S.N., Dmitrovskii A.A., Das R.C. and Ganguly J. (1977) Studies on the metabolism of *beta*-carotene and apo- *beta*-carotenoids in rats and chickens. *Biochimica and Biophysica Acta,* **486**, 183-194.

Simpson K.L. and Chichester C.O. (1981) Metabolism and nutritional significance of carotenoids. *Annual Review of Nutrition,* **1**, 351-374.

Sporn A., Schobeschm O., Marin, V., Pansitescu, E. and Runcan, L. (1965) The toxicity of butyl acetate, methyl naphtyl ketone and ionone. *Igiena*, **XII(5),** 437-446.

Stickley D. P. (1990) Biodegradability of Lilestrialis and gamma-methylionone 600 UC. Report No. 8720. Unpublished report.

Stofberg, J. and Grundschober, F. (1987) The consumption ratio and food predominance of flavoring materials. *Perfumer & Flavoris,t* **12**, 27-56.

Stofberg, J. and Kirschman, J. C. (1985) The consumption ratio of flavoring materials: A mechanism for setting priorities for safety evaluation. *Food and Chemical Toxicology,* **23**, 857-860.

Trent University (1999) Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11. Based on Mackay, Donald (1991) Multimedia environmental models: The fugacity approach. Lewis Publications, CRC, FL..

Vane F.M., Bugge C.J.L., Rodriguez L.C., Rosenberger M. and Doran T.I. (1990) Human biliary metabolites of isotretinoin: Identification, quantification, synthesis and biological activity. *Xenobiotica*, **20**, 193-207.

Wagner V.O. III and Caruthers S.M. (1999) Bacterial Reverse Mutation assay of Methyl Ionone. Unpublished report to RIFM.

Wild D., King, M.T., Gocke, E. and Eckhardt, K. (1983) Study of artificial flavouring substances for mutagenicity in the salmonella/microsome, basc and micronucleus tests. *Fd Chem Toxicol*., **21(6),** 707-719.

Willhite C.C (1986) Structure-activity relationships of retinoids in developmental toxicology. II. Influence of the polyene chain of the vitamin A molecule. *Toxicology and Applied Pharmacology*, **83**, 563-575.

WSKOWIN EPI Suite (2000) US Environmental Protection Agency.

Yu S.G., Anderson, P.J. and Elson, C.E. (1995) Efficacy of beta-ionone in the chemoprevention of rat mammary carcinogenesis. *Journal of Agricultural and Food Chemistry*, **43(8),** 2144-2147.